

EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapour

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ABSTRACT The cerebral effect of long term (mean 15.6, SD 8.9 years) and low (about 25 µg/m³ air) exposure to mercury vapour was studied in a group of 41 workers in a chlor-alkali plant and in a group of matched referents by electroencephalography (EEG). In the visually interpreted EEGs only a tendency for an increased number of EEG abnormalities, especially focal ones, could be seen in the exposed subjects. In the computerised EEG (cEEG), however, the exposed workers had significantly slower and more attenuated EEGs than the referents. This difference was most prominent in the occipital region, became milder parietally, and was almost absent frontally. Our results suggest that cEEG may show early effects on the brain of exposure to mercury vapour.

Symptoms from the central nervous system such as weakened memory, insomnia, dizziness, and tremor have been early findings in workers excessively exposed to metallic mercury (Hg) vapour.^{1,2} In chronic poisoning caused by Hg vapour diffuse slowing of electroencephalograms (EEG) has been shown in five of nine patients by Vroom and Greer.³ Furthermore, toxic effects of Hg on the EEG have been described as bradyrhythmia or diffuse changes.^{4,5} So far as we know, no computerised EEG (cEEG) studies showing the effects of exposure to Hg vapour have been published, even though the method has been frequently applied in pharmaceutical research.⁶

The purpose of our study was to discover whether EEG, and especially cEEG, could show cerebral effects caused by long term low exposure to Hg vapour. This was indeed the case; we found slowing and attenuation of EEG especially in the occipital area of the brain.

Subjects and methods

STUDY GROUP AND STUDY DESIGN

The study had a cross sectional design in which the data from exposed subjects were compared with the data of their matched referents. The indicator of the long term Hg exposure was calculated from the retrospectively collected results of the blood Hg analyses.

The exposed group consisted of 41 men from a chlor-alkali plant with an exposure time of at least five years. Their mean age was 38.1 (SD 6.7, range 28-56) years. The exposure time varied from five to 27 years (mean 15.6, SD 8.9). Subjects with neurological, psychiatric, and metabolic diseases, skull injuries, hypertension, and a history of mercury intoxication were excluded on the basis of previous documents of periodical health examinations performed twice a year as well as of possible case records. Five chlor-alkali workers were excluded from the study, three with hypertension medicated by beta-blockers and two with periodical excessive use of alcohol. One subgroup (n = 21) had regular daywork. The second subgroup (n = 20) had varying three-shift work with periods of four workshifts and two rest days.

One referent, matched for age (± 1 year) and sex, was chosen for every exposed subject. The 41 referents were employed in mechanical wood processing plants. Exclusion criteria from the reference group were earlier occupational exposure either to heavy metals, wood conserving chemicals, or solvents and a history of the diseases or injuries listed above. Rejection from the referent group was based on medical records and on the judgment of the occupational health staff of the employing company. The mean age of the referents was 38.1 (SD 6.6, range 28-55) years. All referents had regular daywork.

EXAMINATIONS

The subjects came at 1300 from their workplaces to Oulu University Central Hospital where the examina-

tions took place. The dayshift workers and referents had had five and the exposed three-shift workers six working hours before their arrival. Only one subject was examined a day. Special attention was given to standardisation of timing and to the strict procedure of the examinations.

At the beginning of the examination every subject was interviewed according to a fixed schedule where previous medical and occupational history, actual health status, and life style were investigated. The interview was followed by a routine clinical examination and a computer administered psychological test battery. After a short rest the examination continued with measurements in the laboratory of clinical neurophysiology. The subjects did not smoke or take coffee during the three hours preceding the EEG recording. Based on the interviews and the observations from the routine clinical examinations, the health state of all the workers was judged to be good. The subjects did not report actual use of any medicines possibly affecting the central nervous system and they had not consumed more alcohol than two bottles of beer ($=0.7$ l) the previous evening.

EXPOSURE DATA

The time weighted average concentration of Hg in blood (the TWA B Hg) was computed as an indicator of long term exposure. Measurements of B Hg covered the period from 1969 until the study. Since 1974 B Hg had been monitored with the cold vapour atomic absorption method of Magos and Cernik⁷ modified by Lajunen *et al.*⁸ Both the inorganic and organic fractions of Hg could be detected by this method.

The dose indicators of the actual exposure of the study group were inorganic B Hg (io B Hg), organic B Hg (o B Hg), total B Hg (B Hg), and urinary Hg (U Hg). A venous blood sample (25 ml) was taken during the hospital examination. Morning urine samples collected at home on the morning of the examination day were used for U Hg analyses. The polyethylene blood tubes (containing 0.15 ml Heparin Medica 5000 U/ml, Finland) and the urine bottles had been

checked for lack of Hg contamination. The modification of Magos and Cernik was applied in analyses of actual Hg concentrations in both blood and urine. The Hg concentration in urine was corrected for a urinary creatinine concentration of 1 mmol/l according to Jaffe's standard method.⁹

CHARACTERISTICS OF THE STUDY GROUP

The individual TWA B Hg concentrations were based on the average of 22 (SD 5.7, range 9–32) measurements. The mean TWA B Hg of the exposed group was 59.0 (SD 12.6, range 40.7–90.0) nmol/l. The mean TWA of inorganic B Hg for the group was 34.9 (SD 12.6, range 17.7–58.5) nmol/l. In nine exposed subjects the TWA organic B Hg concentration exceeded the concentration of the TWA inorganic B Hg.

Table 1 shows the figures of the indicators of actual exposure. For three of the exposed, the organic B Hg was higher than the inorganic fraction. Among the exposed workers the mean concentrations of Hg in blood and urine were rather low compared with earlier published results—for instance, by Skerfving and Berlin.¹⁰ None of the referents had more inorganic than organic Hg in their blood. The blood and urinary Hg concentrations of the reference subjects were also low.

To minimise the possible confounding effect of different workshifts, the exposed three-shift workers attended examinations during their morning shift. When the exposure of the exposed day labourers and three-shift workers were compared, the exposure level of the former group was significantly higher than that of the latter group (fig 1). The TWA B Hg of the day worker group was 65.9 (SD 10.9, range 45.7–82.4) nmol/l and of the three-shift worker group 51.9 (SD 10.2, range 40.7–90.0) nmol/l. The inorganic TWA B Hg of the day workers was also higher, 42.6 (SD 12.6, range 20.4–64.1) nmol/l, compared with the value of the three-shift workers, 26.9 (SD 6.0, range 16.9–37.0) nmol/l.

Table 1 Figures of the indicators for actual exposure of the exposed and reference groups

Indicator of exposure	Exposed (n = 41)			Referents (n = 41)		
	Mean	SD	Range	Mean	SD	Range
io B Hg (nmol/l)	38.8	24.5	10–130	4.3	2.9	< 5–10
o B Hg (nmol/l)	19.1	10.8	10–165	14.5	8.0	5–40
B Hg (nmol/l)	58.0	26.5	25–150	18.8	8.9	5–45
U Hg (μmol/mol) creat (nmol/l)	11.6 (96.6)	7.4 (61.6)	2.1–31.2 (20–260)	1.1 (9.2)	0.8 (6.7)	0.0–2.9 (0.0–25)

io B Hg = Inorganic blood mercury.

o B Hg = Organic blood mercury.

B Hg = Total blood mercury.

U Hg = Urinary mercury.

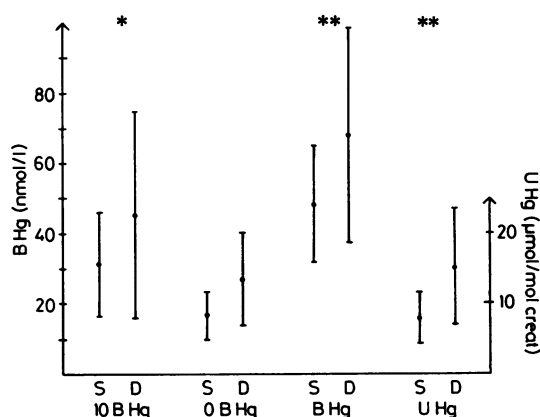


Fig 1 Means and SDS of actual dose indicators of exposed subjects grouped according to workshifts. (S = three-shift workers, $n = 20$; D = day workers, $n = 21$. io B Hg = inorganic blood mercury; o B Hg = organic blood mercury; B Hg = blood mercury; U Hg = urinary mercury. * $p < 0.05$; ** $p < 0.01$).

RECORDING OF EEG

The EEGs were recorded with a 16 channel electroencephalograph. During the recording the subjects lay awake with their eyes closed. Both photic stimulation (0–40 Hz) and hyperventilation (3 min) were performed within every recording. Visual interpretation of the EEGs was performed by one of the authors (UT) without knowledge as to whether the subject belonged to the exposed or reference group. The classification of the normality and abnormality was made according to the scaling presented earlier by Tolonen and Abonen.¹¹ The scaling, including both focal, generalised and paroxysmal EEG disturbances, consisted of four classes: (i) normal, (ii) mildly disturbed, (iii) moderately disturbed, and (iv) severely disturbed.

The recording of the EEG for quantification was always performed in the same way: before each recording session the subject lay with eyes open and talked with the EEG technician to ensure that normal vigilance was maintained. Moreover, if during the recording the EEG showed signs of drowsiness, the EEG technician stimulated the subject to keep awake. For quantification the EEGs were recorded using a time constant of 0.3 s and a high frequency cutoff point of 70 Hz. Only derivations O_1 and O_2 with eyes open and closed, P_3 and F_3 as well as P_4 and F_4 with eyes closed, all referenced to linked ears (A_1A_2), were computerised. For EEG quantification of all subjects, a representative artifact free epoch of 40 (or 20 + 20) seconds from each channel was selected without knowledge of the exposure status of the subject. The EEG quantification was performed off line (from a digital PCM tape recorder) by means of a PDP 11/23

minicomputer. The signal was low pass filtered at 31.5 Hz and the sampling frequency used was 100 Hz. Several power spectral parameters, including both absolute and relative powers, with Fast Fourier Transform were calculated. In addition to absolute and relative band power parameters, some other parameters were calculated, such as mean frequency and also amplitude of the signal reflected by root mean square (RMS). The frequency bands used for calculations were 13.5–30 Hz for beta, 7.5–13.5 Hz for alpha, 3.5–7.5 Hz for theta, and 0.5–3.5 Hz for delta.

Results

VISUALLY INTERPRETED EEG

Table 2 shows the visually interpreted EEG findings for both the study groups. A greater occurrence of EEG abnormalities in the exposed (24%) than in the referents (15%) could be seen, but the difference was not statistically significant. Paroxysmal epileptiform activity was detected in two, both belonging to the exposed group: one had parieto-occipital spikes on the left side and the other had a theta burst occipitally on the right side. In the exposed group focal abnormalities slightly dominated over generalised disturbances but in the reference group generalised EEG disturbances were more abundant. Focal abnormalities were all mild and were situated in the left hemisphere frontotemporally-temporally-temporobasally except in one subject among the exposed group, the focus of whom was parieto-occipitally on the left side. Three subjects had both generalised and focal abnormalities. All EEG abnormalities except one were scaled as "mild." One referent showed a moderate EEG disturbance that appeared as a slowing of background activity imitating poorly matured EEG.

QUANTITATIVE EEG

Firstly, comparisons were made between the exposed and reference groups' power density spectra recorded from the three regions of the brain. The most sig-

Table 2 Visually interpreted EEG findings in the exposed and reference groups

EEG	Exposed		Referents	
	No	%	No	%
Normal	31		76	
Disturbed:	10		35	
Generalised			6	
Focal	4		5	
Paroxysmal	2		2†	
Total	41	100	41	100

*Two subjects had both generalised and focal disturbances.

†One subject had both generalised and focal disturbances.

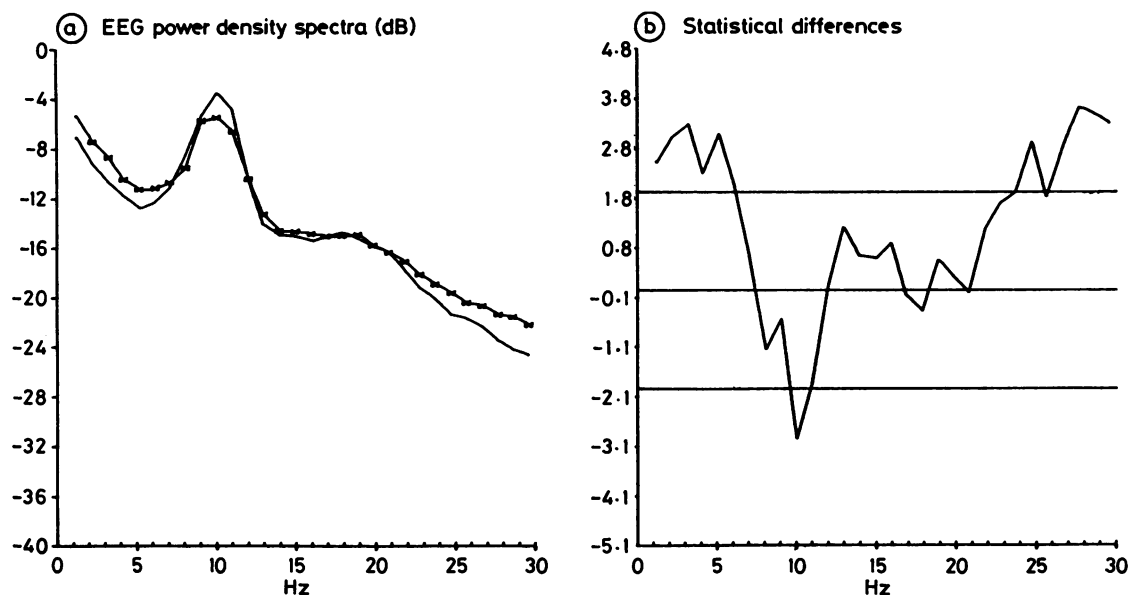


Fig 2 (a) Mean power density spectra of occipital registration ($O_1-A_1A_2$) of exposed (****) and reference (—) groups. (b) Statistical comparison (two sided *t* test with Welch's correction) between exposed and reference groups' spectra. Horizontal lines above and below zero level indicate 95% confidence interval.

nificant difference between the groups was seen occipitally (fig 2). The results from the right and left hemisphere were equal to each other. The difference became milder parietally and almost disappeared frontally. All the single absolute cEEG parameters shown in table 3 were lower in the exposed than in the referents. Highly significant differences ($p < 0.001$) were seen in the alpha frequency powers, total powers, and values of RMS (reflecting the amplitude of the signal) of the spectra.

Secondly, to explore the possible confounding effect of the vigilance on the cEEG of the exposed, the cEEG parameters of the exposed day workers and three-shift workers were compared with the parameters of their

referents (table 4). In the dayworker group all the parameters were lower than in their referents even if a statistically significant difference was seen only in the power of alpha frequency ($t=2.2$, $p < 0.05$). In the three-shift worker group the slowing and attenuation of the cEEG was more emphasised, the difference between all the parameters of this exposed subgroup and their referents being statistically significant. Figure 3 shows the diagrams of the statistical differences between the exposed dayworkers and their referents as well as between the exposed three-shift workers and their referents. In addition, a two way analysis of variance was applied to detect connections between workshift, dose indicators and values of the

Table 3 Absolute frequency powers, total powers, and amplitudes of the signal reflected by root mean square (RMS) in the cEEG for the exposed and reference subjects. (Two sided *t* test with Welch's correction, derivation O_1 referenced to linked ears, eyes closed)

EEG variable	Exposed (n = 41)		Referents (n = 41)		t	p
	Mean	SD	Mean	SD		
Mean frequency (Hz)	8.3	1.3	9.0	1.0	-2.7	<0.01
Frequency (pW):						
Delta	12.9	5.4	16.8	9.6	-2.3	<0.05
Theta	8.7	5.6	14.3	11.9	-2.8	<0.01
Alpha	35.6	36.0	90.5	90.2	-3.6	<0.001
Beta	8.4	5.6	16.1	13.1	-3.4	<0.01
Total power (pW)	65.5	46.0	138	112	-3.8	<0.001
RMS (μ V)	7.7	2.6	11.0	4.3	-4.2	<0.001

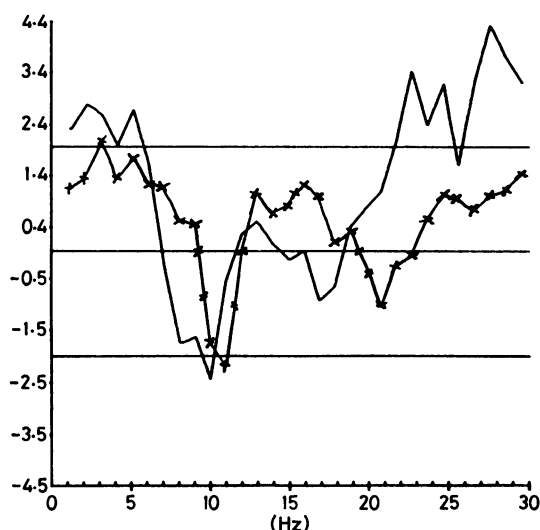


Fig 3 Statistical differences between occipital ($O_1-A_1A_2$) mean power density spectra of cEEG for exposed three-shift workers and their referents (—) and for exposed day workers and their referents (****). Horizontal lines above and below zero indicate 95% confidence interval. (Two sided *t* test with Welch's correction.)

alpha frequency, total power, and signal RMS. No significant relations, however, were seen (all *F* values < 3.07 , $p > 0.09$).

Discussion

In our study a tendency towards increased, mainly focal disturbances was seen in the visually interpreted EEGs of the chlor-alkali workers. Mellerio and Kubicki have described fast activity in the EEG at the outset of mercury intoxications.⁴ In more advanced cases slow rhythms together with paroxysmal activity have been seen. Besides a few case reports, no cross

Table 4 Absolute frequency powers, total powers, and amplitudes of the signal reflected by root mean square (RMS) for the exposed day and three-shift workers. (Derivation O_1 referenced to linked ears, eyes closed)

EEG variable	Day workers (<i>n</i> = 21)		Three-shift workers (<i>n</i> = 20)	
	Mean	SD	Mean	SD
Frequency (pW):				
Delta	12.8	4.3	12.9	6.6
Theta	9.7	6.9	7.6	3.7
Alpha	44.9	37.7	25.8	32.1
Beta	9.6	6.8	7.1	3.7
Total power (pW)	77.0	48.4	53.5	41.1
RMS (μ V)	8.4	2.6	6.9	2.5

sectional or follow up studies have been published concerning EEG findings in workers exposed to Hg vapour. Our data showed some similarities with earlier reported findings, even though the present exposed workers were considered as being without symptoms of Hg intoxications.

Of the subjects in our reference group, 15% showed EEG disturbances. This agreed well with the classic work of Gibbs and Gibbs¹²: in their material of 1000 adults they found EEG abnormalities in about 15% of the subjects when slow and fast activity as well as paroxysmal EEG abnormalities were included. Selected populations such as young male flight personnel have shown an even lower number of EEG abnormalities—namely 7% definitely abnormal and 5% borderline or mildly abnormal.¹³ Although some of the EEG abnormalities reported in these studies are nowadays considered as normal phenomena, our scaling of the EEG findings did not deviate appreciably from the interpretation of the others. Furthermore, mild EEG abnormalities do not necessarily indicate brain disease. Functional changes may occur in the EEG due to, for example, metabolic and toxic causes without structural brain damage.

In the cEEG the difference between the exposed and the referents was most prominent in the recordings from the occipital area. The choice of the frontal, parietal, and occipital areas for recording was made on the knowledge of the uneven accumulation of Hg in the brain cortex, which Takahata *et al* have shown in two necropsy cases with a history of Hg vapour intoxication.¹⁴ The changes in cEEG in the present study were pronounced in the occipital areas and almost disappeared frontally, thus corresponding to the lower accumulation of Hg measured by Takahata *et al*.

The cEEG of the exposed workers showed slowing and attenuation of the power density spectra in comparison with the referents. In our study the shiftwork was an obvious confounding factor. Even though special attention was paid to the standardisation of the timing of the examinations, the one hour earlier start of the workday as well as the strain due to changing workshifts might have caused more fatigue in the three-shift worker group, and thus decrease of their vigilance. The cEEG has been used as one of the best measures of vigilance.¹⁵ Indeed, the findings of the three-shift workers were clearly emphasised. Nevertheless, the overall attenuation of the cEEG remained in the dayworker group. Besides, there was no reason to suspect a similar difference in vigilance between the dayworkers and the referents, which was the case among the three-shift workers.

Another confounding effect could have arisen from exposure to methyl Hg from the general environment. The brain has shown a special ability to accumulate

both elementary Hg vapour and methyl Hg.^{5,16} In another forthcoming part of this present study it was shown that the magnitude of the organic B Hg fraction of both exposed and reference subjects was connected to their consumption of fish. The most exposed chlor-alkali workers had lately restricted their fish meals to lessen their overall exposure, but among the exposed and the referent groups were subjects with a generous consumption of fish. Unfortunately, because no indicator of the methyl Hg dose in the brain caused by low long term exposure has been developed, the possible influence of additional exposure to methyl Hg on the cEEG could not be controlled in the study.

No suggestion of a dose effect relation was found in this study. The obvious explanation is that the concentrations of B Hg or U Hg and the concentrations of Hg in the brain do not correlate in general because they measure different temporal exposure.^{10,17}

Based on the dose indicators applied in this study, the exposure level of both the chlor-alkali workers and the reference workers was low. Based on the ratio of concentrations of Hg in air and blood presented by Roels *et al.*,¹⁸ the long term average Hg concentration in air had probably been almost equal to WHO's health based recommended level of 25 µg/m³.¹⁷ This estimation, however, was not quite accurate because of the short biological half time of inorganic Hg in blood.¹⁹ The estimation of the exposure level during the examinations was therefore more reliable. The dayworkers showed an average exposure of about 25 µg/m³ of air and the three-shift workers about 15 µg/m³ of air.

We conclude that the slowing and attenuation of the cEEG observed in the exposed workers was related to the low long term exposure to Hg vapour. The mental strain caused by shiftwork accentuated the disturbance in the EEGs. A longitudinal study design would be needed to verify these suggestive results. The computerised EEG could be developed into a method for measuring early neurological effects of exposure to Hg vapour.

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